(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 March 2001 (08.03.2001)

PCT

(10) International Publication Number
WO 01/15753 A1

(51) International Patent Classification[7]: A61L 27/40, 27/38

(21) International Application Number: PCT/EP00/08157

(22) International Filing Date: 22 August 2000 (22.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
99402149.1 30 August 1999 (30.08.1999) EP

(71) Applicant *(for all designated States except US)*: **I.D.M. IMMUNO-DESIGNED MOLECULES** [FR/FR]; 172, rue de Charonne, F-75011 Paris (FR).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **BARTHOLEYNS, Jacques** [FR/FR]; Manoir de la Vignole, 1, impasse Marguerite d'Anjou, F-49730 Turquant (FR).

(74) Agents: **GROSSET-FOURNIER, Chantal** et al.; Grosset-Fournier & Demachy SARL, 20, rue de Maubeuge, F-75009 Paris (FR).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/15753 A1

(54) Title: NEW HUMANIZED BIOMATERIALS, A PROCESS FOR THEIR PREPARATION AND THEIR APPLICATIONS

(57) Abstract: The invention relates to humanized biomaterial comprising a porous biocompatible composite material customized and implanted with monocyte derived cells preferably with macrophages.

NEW HUMANIZED BIOMATERIALS, A PROCESS FOR THEIR PREPARATION AND THEIR APPLICATIONS

The invention relates to new humanized biomaterials, a process for their preparation and their applications.

Tissue repair is needed after severe bone fractures, cartilage loss or general fragilization during ageing.

Artificial metallic or even ceramic prostheses are not very well integrated within host tissues and replacement with new surgery is often required after a few years, a major problem of handicap for old people.

Autologous grafts of bones or cartilage tissue is very difficult and costly. New technologies develop porous matrices implanted as scaffold prosthesis.

These matrices can eventually be filled with growth factors for the tissue regeneration or even with bone marrow stem cells.

However, fixation of cells or factors in the porous matrices with very prolonged and slow release of growth factors is very difficult to achieve and the ideal cocktail and concentration of factors required is unknown.

The aim of the present invention is to provide a homogeneous humanized, bioactive biomaterial (for example porous ceramics) that can be used for implantation purposes and which do not present the long term biocompatibility problems of prior art.

Another aim of the invention is to provide a bioactive biomaterial enabling tissue growth (for example bone and cartilage) in its porous space and securing the integration of the grafted biomaterial in the surrounding tissues (viable bones...).

Another aim of the invention is to provide long lasting prostheses, which avoids requirement for replacement of biomaterial prostheses after 10 years, as often needed up to now.

These aims are achieved by the invention, which consists in humanized biomaterial comprising a porous biocompatible composite material customized and implanted with monocyte derived cells and preferably with macrophages.

The expression "humanized" means that the porous biomaterial has been colonized with human cells derived from blood monocytes.

The expression "biocompatible composite" material designates a material composed of one or several of the following materials proved to be non toxic for human tissues (carbon microfibers, ceramics, calcium phosphates, metal oxides, collagen polymers...).

The expression "porous" means that the biomaterial and preferably the ceramic present pores of about 100 to 2000 microns of diameter.

The expression "customized and implanted" material means that the shape and size of biomaterial is designed specifically for a patient and a site of implantation.

The expression "monocyte derived cells" corresponds to human mononuclear cells isolated from blood, enriched in monocytes and cultured at 37° C in appropriate medium, for 5 to 10 days to obtain tissue type macrophages.

The monocyte derived cells used in the invention are for instance such as those described in PCT/EP 93/01232, WO 99/13054, EP 96/ 901848.0-2107, WO 97/44441.

In a particular embodiment of the invention, the monocyte derived cells described above, contain exogenous compounds such as drugs, proteins, growth factors of interest.

In another embodiment, the monocyte derived cells as described above contain in their cytoplasm exogenous DNA coding for a protein of interest.

The substantially irreversible humanization of matrices of biocompatible composite material described in the present invention allows a physiological interaction between the prostheses made of the biomedical composite, grafted and the host cells in the body. These relations with host tissue cells and with the extracellular matrix allow reconstruction of epithelial sheets and growth of a capillary network around the grafted biomaterial by local multiplication and sprouting of endothelial cells.

The monocyte derived cells, in particular the macrophages used to humanize *in vitro* the porous material in the invention are particularly adequate to increase integration and *in vivo* lifespan of biocompatible prostheses.

Advantageously, the humanized biomaterial of the invention is homogenous.

According to an advantageous embodiment, in the humanized biomaterial of the invention, the biocompatible composite material is chosen among the following materials : microfibres, ceramic materials, metal oxides such as aluminum oxide, calcium phosphate ceramic, glass or carbon fibers, hydroxylapatite, silicon carbide or nitride, collagen polymers or a mixture of these different materials.

According to another advantageous embodiment, in the humanized biomaterial of the invention, the human macrophages are liable to be obtained by *ex vivo* differentiation from blood monocytes leading to living macrophages, and are cultured under conditions enabling their penetration and adherence into the biomaterial for instance for several hours at 37°C, with the porous biomaterial, allowing infiltration of the biomaterial and substantially irreversible binding of the living macrophages to

the biomaterial, now humanized with patient's macrophages and ready for implantation.

The expression "substantially irreversible binding" means that macrophages are tightly bound by numerous contacts with the material and cannot be detached under physiological conditions.

The invention also relates to a living body-supporting implant, characterized by the fact that it comprises or consists of the humanized biomaterial according to any one of claims 1 to 3, and is preferably structured under the form of scaffold, tissue-supporting sponges, bone or cartilage.

The expression "living body-supporting implant" designates an implant having for example the physical form and robustness of a bone to be replaced.

The expression "scaffold" designates a physical structure eventually metallic which keeps the biomaterial (ceramics or other) in the appropriate conformation.

The expression "tissue supporting sponges" designates a soft implant formed for instance of collagen which will be humanized with macrophages before insertion in the body.

The invention also relates to the use of a humanized biomaterial of the invention or of a living body-supporting implant of the invention, for the preparation of a tissue implant destined to replace or repair defective tissue, such as defective bone, cartilage, dental tissue, fibrous tissue, fibrocartilaginous supporting tissue.

The expression "fibrous tissue" designates tissues surrounding organs which support this organ and maintain the shape of this body part : they are mainly formed by epithelial sheets.

According to another advantageous embodiment, the invention relates to the use of a humanized biomaterial of the invention or of a living body-supporting implant of the invention, wherein the monocyte derived cells or macrophages are autologous with respect to the tissue to be replaced or repaired, enabling the biomaterial or the living body-supporting implant to be recognized as self.

The expression "implant to be recognized as self" means that it contains cells (i.e. macrophages) from the patient in which it will be grafted and is therefore autologous to the host.

A process for the preparation of a humanized biomaterial of the invention comprises the following steps :

- preparation of the porous biomaterial structured in form of bones, cartilage,
- preparation of macrophages from blood monocytes,

- immersion of the biomaterial in a physiologic solution appropriate for the culture of macrophages which are added afterwards (ex. : phosphate buffered saline, medium such as RPMI, IMDM, AIMV),
- addition of the macrophage to the solution under conditions enabling binding to the biomaterial and particularly for 1 to 20 h. at 37°C, 5 % CO2 and 5 % air,
- washing of the biomaterial and conservation until use in physiologic medium.

A process for the preparation of a living body-supporting implant of the invention comprises the following steps :

- preparation of a customized porous implant or scaffold composed of bio-compatible material, according to any one of claims 1 to 3,
- preparation of macrophages from blood monocytes of the patient needing the customized implant of biomaterial,
- co-culture of macrophages and the implant in adequate medium under conditions enabling penetration and adherence to the biomaterial, in particular at 37°C, 5% CO2 in hydrophobic bags or containers until grafting the implant.

The invention also relates to the use of the humanized biomaterial or of a living body-supporting implant, which can be implanted in a tissue, for the *in vitro* or *in vivo* or *ex vivo* delivery of factors chosen in the group of chemokines and/or monokines, and/or cytokines and/or growth factors, the factors released being useful for the local attraction of cells required for tissue growth (such as osteoblasts, chondrocytes, fibroblasts, epithelial cells.....) and/or for the neovascularization around the implanted biomaterial, and/or for the release of growth factors sustaining proliferation of cells and/or the growth of new tissues.

Indeed, macrophages maintain tissue homeostasis through the secretion of at least 80 growth factors or monokines controlling and inducing proliferation of mesenchymal (fibroblasts....), endothelial, chondrocytes, osteoblasts, **epithelial** cells.. They also secrete enzymes and mediators allowing growth and renewal of the surrounding cells and tissues (see Table 1).

The key factors secreted by macrophages supporting tissue integration regeneration and growth of mesenchymal cells are : IGF1 and TGFs, but also PDGF, bFGF, MDGF, GM, CSF, NAF, IL-8, TNF, angiogenin and angiogenic factors. These growth factors allow also the development of all the steps required for angiogenesis, allowing neovascularisation and reconstitution of blood microcapillaries around the grafted biomaterial.

In this aspect, macrophages are synthesizing 10 fold more proteins than monocytes, much more growth factors and less inflammatory mediators.

TABLE 1 :

GROWTH FACTORS PROTEINS AND MEDIATORS SUPPORTING TISSUE HOMEOSTASICS SECRETED BY NATURE MACROPHAGES

ENZYMES :

Lyzosymes

Neutral proteases
- Plasminogen activator
- Collagenase
- Elastase
- Angiotensin-convertase

Acid hydrolases
- Proteases
- Lipases
- Ribonucleases
- Phosphatases
- Glycosidases
- Sulphatases

Arginase

COMPLEMENT COMPONENTS
C1, 4, 2, 3 and 5
Factors B and D and Properdin
C1 inhibitor
C3b inactivator and β-1H

ENZYME INHIBITORS
(Antiproteases)

α1-antiprotease
Plasmin inhibitors
α-2 macroglobulin
Plasminogen activator inhibitors

PROTEINS BINDING METABOLIOUS AND LIPIDS :
Acidic isoferritins

BIOACTIVE LIPIDS :

Arachidonic acid metabolites
- Prostaglandins E2, F2α
- Prostacyclin
- Thromboxane
- Leukotrienes B4, C, D and E
- Hydroxy-eicosatetraneoic acids (including SRS-A)

Platelet activating factors

CYTOKINES, HORMONES, GROWTH FACTORS :
- Interleukins 1 α and β
- Tumours necrosis factor α
- Interferons α and β_1, &
- Interleukin 6, 8, 13, 18

Chemotatic factors for
- Neutrophils
- Tlymphocytes
- Monocytes
- Fibroblasts

Heamatopoceitic Colony Stimulating Factors for
- Granulocyte-Macrophages (GM-CSF)
- Granulocytes (G-CSF)
- Macrophages (M-CSF)
- Erythropoeitin

Growth factors
- Fibroblasts growth factor / Insuline like G.F.
- "platgelet-derived growth factor" (PDGF)
- Transforming growth factor α and β
- Endothelial cell growth factor

Hormones
- 1 α, 25-Dihydroxyvitamin D3

Transferrin
Transcobalamin II
Fibronectin
Laminin
Lipid transfer protein
Thrombospondin

NUCLEOSIDES AND METABOLITES :
Thymidine and deoxycytidine
Uracil
Uric acid
Lactic acid
Polyamines nitrines and nitrates
Neopterin

Insulin-like activity, protagandins
Thymosin B4
β endorphin
Adrenocorticotrophic hormone

CHEMOKINES MIP / RANTE FAMILIES
COAGULATION FACTORS :
Factors III, VIII, and tissue factor
Prothrombin and prothrombinase
Factors IX, X, V and VII

In an advantageous embodiment of the invention, the macrophages migrate initially in the porous biomaterial and incorporate irreversibly into this prosthesis by very strong adherence and spreading. When they are kept in physiological conditions, macrophages are very long living cells lasting from several months to several years after implantation. During this time, macrophages will continuously secrete growth factors and cytokines in their local environment ; these factors will act in synergy at very low concentrations (10^{-10} M) on the surrounding cells and tissues.

Furthermore, macrophages do present on their membranes receptors for cytokines, hormones, sugars allowing to respond to micro-environmental needs and to adjust their secretion to the local status around the biomaterial at different periods after grafting.

The growth factors secreted by macrophages represent the global requirements for tissue repair, differentiation and local angiogenesis. The chemokines which will be continuously released in a concentration gradient around the implant will call in and around the prosthesis cells required for recolonization and integration of the biomaterial in host environment. Therefore, the new customized porous biomaterials colonized with host macrophages present a novel biotolerance and a length of adequate performance far better than prosthesis used in the absence of autologous macrophages. Applications are very large in solid or cartilaginous prosthesis needed in bone, cartilaginous repair particularly.

According to another advantageous embodiment, the invention relates to the use of the humanized biomaterial or of a living body-supporting implant, as a graft for the replacement of supporting tissues such as bones, cartilages, dental tissues, epithelial sheet and subcutaneous tissue matrix.

Example 1 :

A calcium phosphate porous ceramic with pores of 200 to 2000 microns (porosity > 20 % and < 80 %) is placed on an hydrophobic support (ethylene vinyl acetate) in the presence of 50 ml AIMV culture medium (life-cell Gibco, Paisley G.B.). Macrophages are added to this preparation at the concentration of 5.10^6 cells/ml ; they are obtained after 7 days differentiation of blood monocytes in culture according to published state of the art in publications and patents (PCT/EP 93/01232, WO 99/13054, EP 96/ 901848.0-2107, WO 97/44441) ; a control preparation is maintained in the absence of macrophages. The preparation is incubated overnight at 37 °C, 5 % CO2, 95 % air to allow fixation of macrophages on the ceramic.

The porous ceramic is washed and cultured in the presence of fibroblasts and/or in the presence of chondrocytes In both cases, the cell proliferation is higher by a factor 2 to 10 for the porous ceramic colonized with macrophages, compared to control ceramic.

Example 2 :

A small fragment of porous microceramic is implanted in a rabbit cornea. The inert microceramic piece induces a very small to moderate inflammation and only peripheral growth of new blood vessels from the ring of the cornea.

In contrast, microceramic covered with macrophages, as described in example 1 induces a major neovascularisation towards the center of the cornea. The cornea becomes vascularized through an invasion of endothelial cells arising from the rim rich in blood supply and sprouting towards the biomaterial implanted.

Example 3 :

Fragments of 100 +/- 20 mg of hydroxyapatite ceramic (Endobon®, Merck) and pieces of one cm2 of a polypropylene scaffold are prepared. Fresh non activated macrophages obtained after 7 days differentiation of blood monocytes in culture according to published patent applications (WO94/26875, WO 99/13054, WO96/22781, WO 97/44441) are suspended (2,5.106 cells/ml) in IMDM (Iscove Modified Dulbecco Medium) culture medium. Each biomaterial fragment is incubated in 1 ml of macrophage suspension, in sterile polypropylene tubes, for 4 hours at room temperature. To check the binding of macrophages on the biomaterial, cells present in the supernatant after incubation are counted. After incubation with Endobon®, from 12 to 17% of the cells added were present in the supernatant (3 experiments),

indicating that more than 2.10^6 macrophages are adsorbed on 100 mg of porous ceramic. After incubation with polypropylene scaffold, from 23 to 55 % of the cells initially added are present in the supernatant, indicating adsorption of 1 to 2.10^6 macrophages/cm2 scaffold.

Nude mice are implanted with biomaterial, and each mouse receives two implants of the same type, one in each flank.

Mice n°	Implanted material
1, 2	Endobon® colonized by macrophages
3	Endobon®
4, 5	Polypropylene colonized by macrophages
6	Polypropylene

Mice are sacrificed after 21 days, macroscopic observation shows no major difference between implants of biomaterial alone and implants of biomaterial colonized by macrophages.

Microscopic observation of tissues in paraffin shows that, when compared to implants of biomaterial alone, implants of biomaterial colonized by macrophages induce first an inflammation phenomenon, which is an important step to induce migration and homing of competent cells for tissue repair. A more important neovascularisation at the implantation site of biomaterial colonized with macrophages has also been observed.

The histological analysis of tissues in resin confirms the increase of neovascularisation for mice implanted with macrophages colonized biomaterials ; when compared to mice implanted only with biomaterial.

The therapeutic applications for tissue repair are confirmed in human bearing non healing ulcers. The ulcers covered with scaffold implanted with autologous macrophages show an improved cicatrization as measured by detersion and size of the ulcer.

CLAIMS

1 - Humanized biomaterial comprising a porous biocompatible composite material customized and implanted with monocyte derived cells and preferably with macrophages.

2 - Humanized biomaterial according to claim 1, wherein the biocompatible composite material is chosen among the following materials : microfibers, ceramic materials, metal oxides such as aluminum oxide, calcium phosphate ceramic, glass or carbon fibers, hydroxylapatite, silicon carbide or nitride, collagen polymers or a mixture of these different materials.

3 - Humanized biomaterial according to claims 1 or 2, wherein the human macrophages are liable to be obtained by *ex vivo* differentiation from blood monocytes leading to living macrophages, and are cultured under conditions enabling their penetration and adherence into the biomaterial, for instance for several hours at 37°C, with the porous biomaterial, allowing infiltration of the biomaterial and substantially irreversible binding of the living macrophages to the biomaterial, being humanized with patient's macrophages and ready for implantation.

4 - Living body-supporting implant, characterized by the fact that it comprises or consists of the humanized biomaterial according to any one of claims 1 to 3, and is preferably structured under the form of scaffold, tissue-supporting sponges, bone or cartilage.

5 - Use of a humanized biomaterial according to any one claims 1 to 3 or of a living body-supporting implant according to claim 4, for the preparation of a tissue implant destined to replace or repair defective tissue, such as defective bone, cartilage, dental tissue, fibrous tissue, fibrocartilaginous supporting tissue.

6 - Use of a humanized biomaterial according to any one of claims 1 to 3 or of a living body-supporting implant according to claim 4, wherein the monocyte derived cells or macrophages are autologous with respect to the tissue to be replaced or repaired, enabling the biomaterial or the living body-supporting implant to be recognized as self.

7 – Process for the preparation of a humanized biomaterial according to any one claims 1 to 3, comprising the following steps :

- preparation of the porous biomaterial structured in form of bones, cartilage,
- preparation of macrophages from blood monocytes,
- immersion of the biomaterial in a physiologic solution appropriate for the culture of macrophages which are added afterwards (ex. : phosphate buffered saline, medium such as RPMI, IMDM, AIMV),
- addition of the macrophages to the solution under conditions enabling binding to biomaterial and particularly for 1 to 20 h. at 37°C,
  5 % CO2 and 5 % air,
- washing of the biomaterial and conservation until use in physiologic medium.

8 – Process for the preparation of a living body-supporting implant according to claim 4, comprising the following steps :

- preparation of a customized porous implant or scaffold composed of bio-compatible material, according to any one of claims 1 to 3,
- preparation of macrophages from blood monocytes of the patient needing the customized implant of biomaterial,
- co-culture of macrophages and the implant in adequate medium under conditions enabling penetration and adherence to the biomaterial in particular at 37°C, 5% CO2 in hydrophobic bags or containers until grafting the implant.

9 – Use of the humanized biomaterial according to any one of claims 1 to 3 or of a living body-supporting implant according to claim 4, which can be implanted in a tissue, for the *in vitro* or *in vivo* or *ex vivo* delivery of factors chosen in the group of chemokines and/or monokines, and/or cytokines and/or growth factors, the factors released being useful for the local attraction of cells required for tissue growth (such as osteoblasts, chondrocytes, fibroblasts, epithelial cells.....) and/or for the neovascularization around the implanted biomaterial, and/or for the release of growth factors sustaining proliferation of cells and/or the growth of new tissues.

10 - Use of the humanized biomaterial according to any one of claims 1 to 3 or of a living body-supporting implant according to claim 4, as a graft for the replacement of supporting tissues such as bones, cartilages, dental tissues, epithelial sheet and subcutaneous tissue matrix.

INTERNATIONAL SEARCH REPORT

Interi nal Application No
PCT/EP 00/08157

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L27/40 A61L27/38

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 902 741 A (DUNKELMAN NOUSHIN ET AL) 11 May 1999 (1999-05-11) abstract column 8, line 59 -column 9, line 57 column 10, line 63 -column 11, line 9 column 13, line 12 - line 40 ---	1,2,4-10
X	US 5 885 829 A (RUTHERFORD ROBERT B ET AL) 23 March 1999 (1999-03-23) column 3, line 64 -column 4, line 31 column 5, line 56 -column 6, line 9 column 11, line 16 - line 65 column 13, line 20 - line 67 ---	1,2,4-10
X	DE 38 10 803 A (BATTELLE INSTITUT E V) 12 October 1989 (1989-10-12) column 1, line 25 -column 2, line 13 column 2, line 67 -column 3, line 33 --- -/--	1-5,7-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 November 2000	05/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Menidjel, R

1

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 00/08157

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 485 096 A (BELL EUGENE) 27 November 1984 (1984-11-27) column 1, line 37 -column 2, line 6 column 2, line 67 -column 3, line 64 column 4, line 31 - line 54	1,2,4-8
X	EP 0 798 374 A (MATRIX MEDICAL B V) 1 October 1997 (1997-10-01) column 2, line 6 -column 3, line 31	1,2,4-8

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/EP 00/08157

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5902741 A	11-05-1999	US 5443950 A	22-08-1995
		US 5266480 A	30-11-1993
		US 5032508 A	16-07-1991
		US 4963489 A	16-10-1990
		US 4721096 A	26-01-1988
		AU 689605 B	02-04-1998
		AU 2769695 A	04-01-1996
		CA 2192064 A	14-12-1995
		EP 0812351 A	17-12-1997
		NZ 288467 A	28-10-1998
		WO 9533821 A	14-12-1995
		US 5962325 A	05-10-1999
		US 6140039 A	31-10-2000
		US 6022743 A	08-02-2000
		US 5863531 A	26-01-1999
		US 5460939 A	24-10-1995
		US 5510254 A	23-04-1996
		US 5580781 A	03-12-1996
		US 5516680 A	14-05-1996
		US 5512475 A	30-04-1996
		US 5541107 A	30-07-1996
		US 5516681 A	14-05-1996
		US 5578485 A	26-11-1996
		US 5785964 A	28-07-1998
		US 5518915 A	21-05-1996
		US 5624840 A	29-04-1997
		US 5849588 A	15-12-1998
		US 5858721 A	12-01-1999
		AU 644578 B	16-12-1993
		AU 4211489 A	02-04-1990
		BR 8907642 A	20-08-1991
		CA 1335657 A	23-05-1995
		DK 40591 A	07-05-1991
		EP 0358506 A	14-03-1990
		HU 56393 A	28-08-1991
		IL 91536 A	31-10-1996
		JP 4501657 T	26-03-1992
		KR 156571 B	15-10-1998
		KR 156684 B	15-10-1998
		KR 156685 B	15-10-1998
		NO 910787 A	22-04-1991
		NZ 230572 A	23-12-1993
		PT 91676 A	30-03-1990
		WO 9002796 A	22-03-1990
		US 5160490 A	03-11-1992
		ZA 8906886 A	27-06-1990
		AT 127692 T	15-09-1995
		AU 6815990 A	14-03-1991
US 5885829 A	23-03-1999	AU 3214797 A	05-01-1998
		EP 0915967 A	19-05-1999
		WO 9745533 A	04-12-1997
DE 3810803 A	12-10-1989	NONE	
US 4485096 A	27-11-1984	AT 25193 T	15-02-1987
		DE 3369465 D	05-03-1987
		EP 0110966 A	20-06-1984

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1,9 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inten nal Application No
PCT/EP 00/08157

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4485096 A		WO 8304177 A	08-12-1983
		US 4485097 A	27-11-1984
EP 0798374 A	01-10-1997	CA 2198978 A	01-09-1997